GENVEC

"Comprehensive Characterization of the 293-ORF6 Cell Line

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GenVec, Inc.

- GenVec is a clinical stage biopharmaceutical company developing therapeutics for the treatment of major diseases, such as cancer, cardiovascular disease and macular degeneration.
- GenVec uses its patented adenovector technology to deliver medically relevant proteins, such as TNF-alpha, VEGF, and PEDF, directly to the site of disease.
- GenVec's adenovector technology is also being used to develop vaccines for infectious diseases.

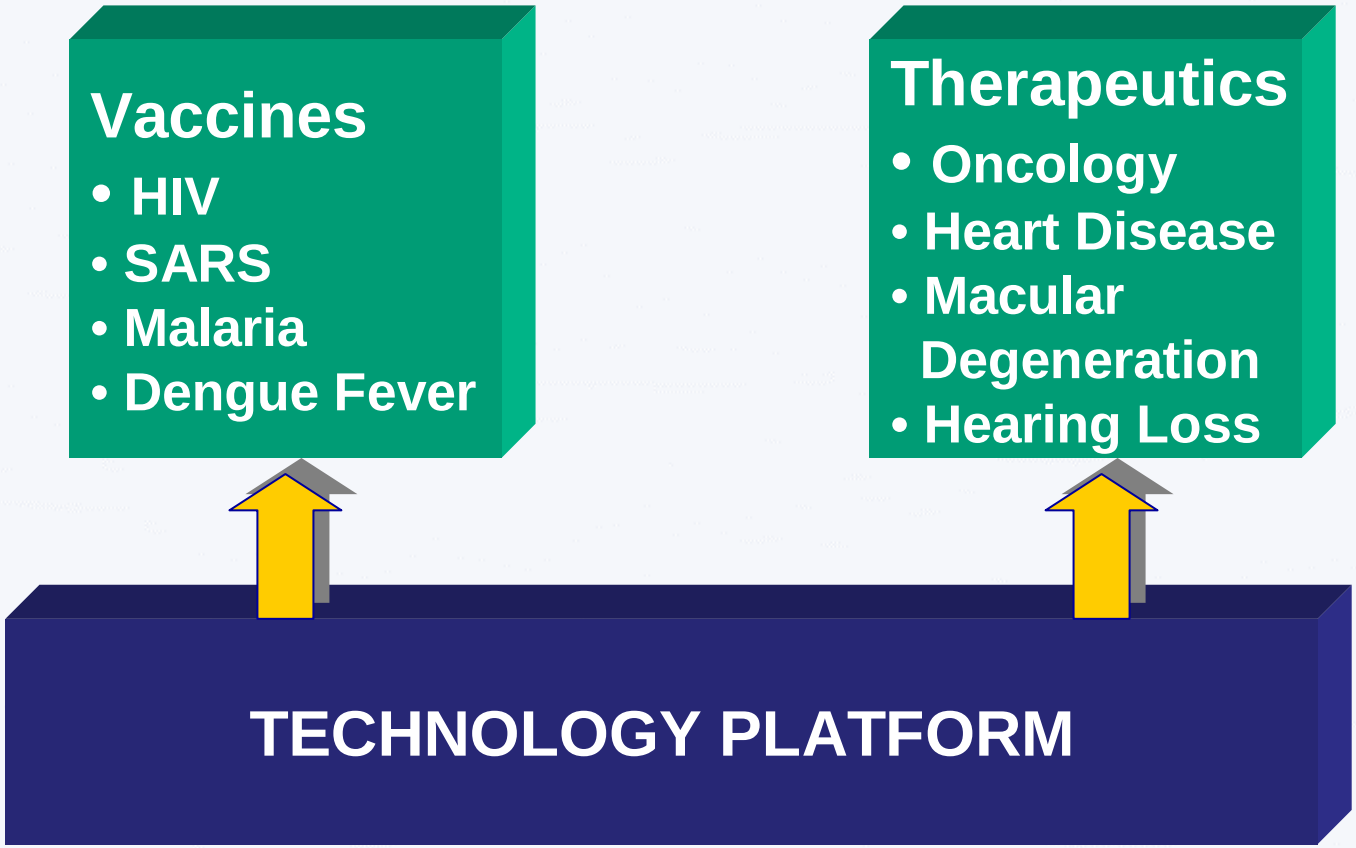
GenVec's Pipeline

- Therapeutic product candidates:
  - Oncology: TNFerade for pancreatic and esophageal cancers - **TNFα**
  - Heart Disease: BIOBYPASS for severe coronary artery disease – **$VEGF_{121}$**
  - Ophthalmology: AdPEDF for wet age-related macular degeneration – **PEDF**

Vaccine Programs

- Collaboration with NIH/VRC for **HIV** and **SARS**
- Collaboration with U.S. Navy for **Malaria** and **Dengue Virus**

Multiple Product Opportunities

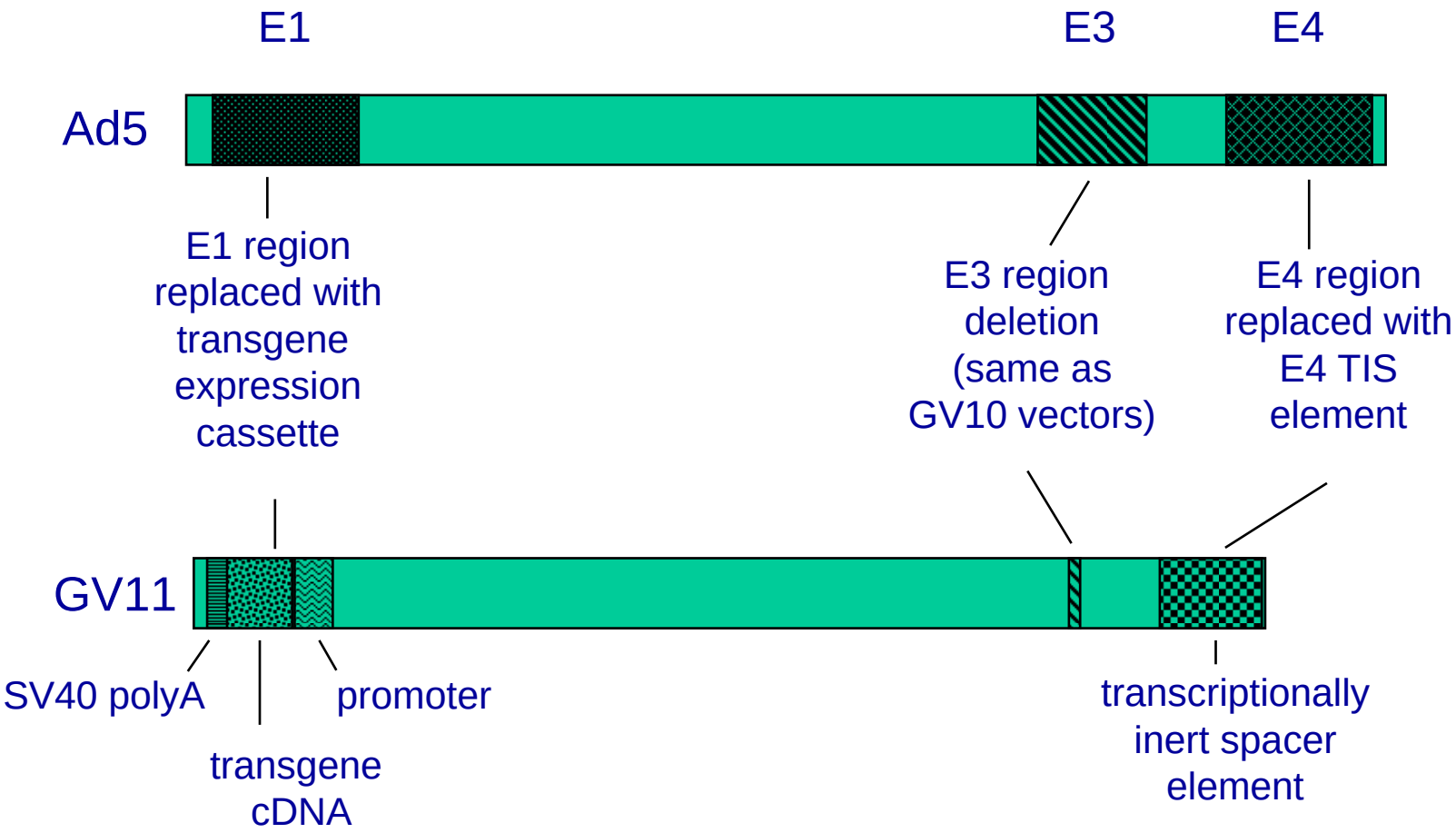


Core Expertise

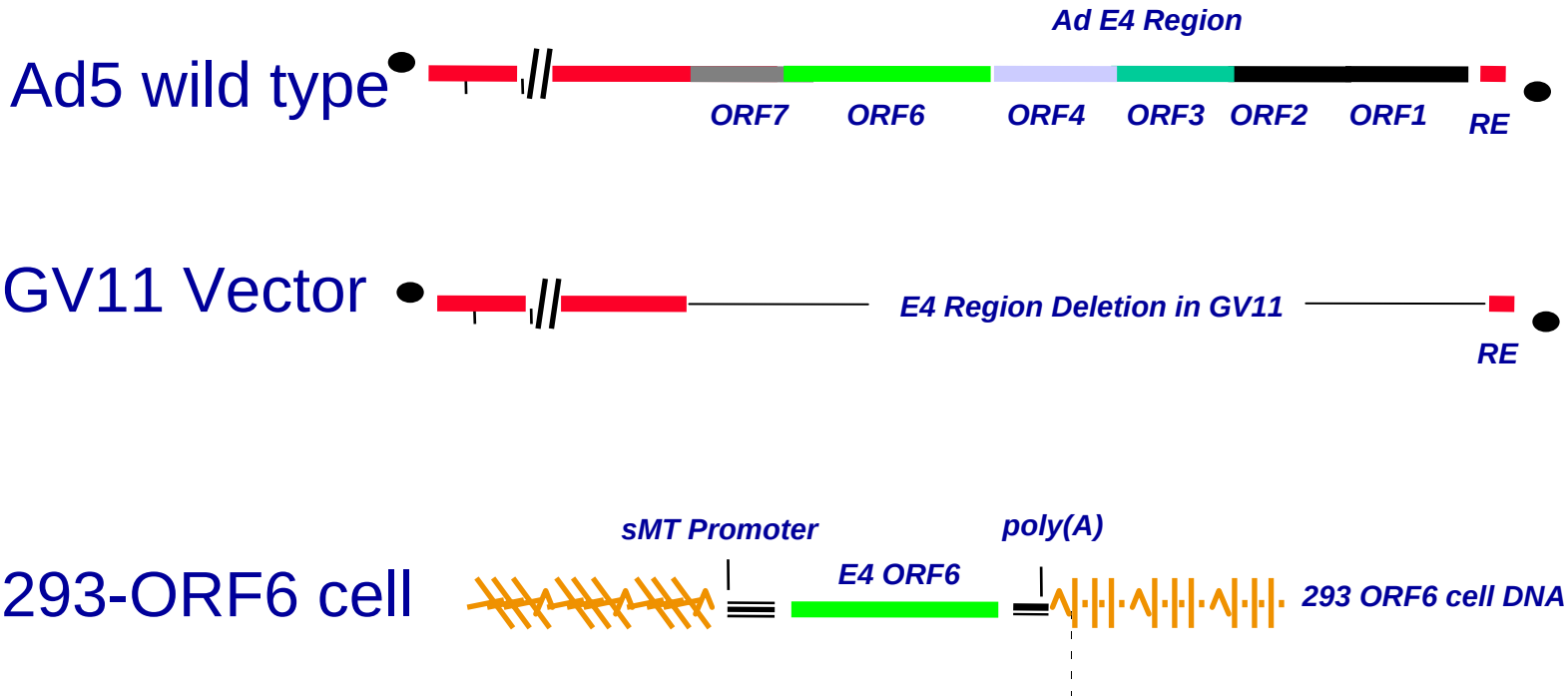
GenVec's Technology

- Replication-deficient Adenovirus vector technology
- "GV11" vector contains deletions in E1, E3 and E4 domains to assure replication incompetence
- Transgene inserted in place of E1 domain for protein production/delivery
- 293-ORF6 cell line developed to provide complementary E1 and E4 sequences

GV11(E1/E3/E4 Deleted) Vector



GenVec's 293-ORF6 Cell Line



- No overlapping sequences exist in the E4 regions of GV11 (E1/E4 deleted) vectors and 293-ORF6 cell line

293-ORF6 MCB Characterization

- Viral and microbial safety panel
- Cytogenetic analysis
- E1 and E4 copy number
- E1 and E4 chromosome localization
- PrP gene sequence
- PrP gene product protease sensitivity
- Tumorigenicity and Oncogenicity
- PCR identity assay

Safety Assessment of MCB

- **Microbial**: Mycoplasma, Sterility (B&F)
- **In Vitro**: Adventitious viruses (3 cell lines), Bovine Viruses (9 CFR), Porcine Parvovirus
- **In Vivo**: Inapparent viruses in animals
- **PCR**: AAV, CMV, EBV, HBV, HCV (RT-PCR), HHV-6/7/8, HIV-1/2, HPV, HTLV-I/II, Parvo B19, SV40, F-PERT (Retroviruses)
- **Biochemical**: Isoenzyme analysis (human origin)
- **Other**: TEM

Cell Aging Study

- "Aged Cell Bank" established to assess cell line stability
  - "P43" designation
    - 16 passages beyond the MCB (P27)
    - Represents theoretical expansion of WCB to 10,000L bioreactor stage
  - P43 bank cryopreserved for characterization studies

Characterization of MCB

- P27 (MCB) compared to P43 (aged cell bank) for the following characterization studies:
  - Cytogenetic analysis (ploidy)
  - Copy number of E1 and E4 insertions
  - Chromosome localization of E1 and E4 insertions
  - DNA sequence of PrP gene
  - Protease sensitivity of PrP gene product
  - PCR identity assay for 293-ORF6 cells

Cytogenetic Analysis

- P27 and P43 cells were expanded, treated with Colcemid, hypotonically lysed, fixed and then stained with Wright's reagent
- 100 cells in metaphase were examined for chromosome count
- Structural abnormalities and chromosome number were recorded
- Abnormalities classified as:
  - Chromatid-type aberrations
  - Chromosome-type aberrations
  - Severely damaged cells

Cytogenetic Analysis

Results:

	P27	P43
Median#/Metaphase	72	72
Range	66-80	54-78
Aberrations/cell	0.02	0.00

E1 and E4 Copy Number

- Q-PCR assay developed
  - E1 assay targeted Ad5 bases 3343 to 3411
  - E4 assay targeted Ad5 bases 34007 to 34073 (specific for ORF6 region)
  - Human GAPDH used as an index for cell number

E1 and E4 Copy Number

Results:

	P27	P43
E1 Copy #	6.5 ± 0.8	5.1 ± 0.7
E4 Copy #	2.6 ± 0.2	2.4 ± 0.4

E1 and E4 Localization

- FISH assay developed to detect E1 and E4 chromosome localization
  - Plasmids encoding full E1 region or the E4-ORF6 expression cassette were labeled with Texas Red 5'UTP by nick translation
  - P27 and P43 cells were expanded, treated with Colcemid, lysed, fixed and then stained with labeled probes and counterstained with DAPI
- Cells in metaphase were analyzed for the presence of red fluorescent signal
- Chromosomes were identified by DAPI

E1 and E4 Localization

Results:

	P27	P43
E1 Local.	1 copy on #6 at 6q16-21	1 copy on #6 at 6q16-21
E4 Local.	2 copies on #19 at 19q13.3	2 copies on #19 at 19q13.3

PrP Gene Sequence

- Prion protein (PrP) open reading frame found on chromosome 20 was sequenced directly from total DNA
- PCR Primers:
  - AP-1 – Palmer, et al, 1996
  - AP-2 – Windl, et al, 1996
- Resultant amplicon was purified and sequenced

PrP Gene Sequence

- Expected 894 bp amplicon coding for 245 aa Prion protein, based on GenBank accession #U29185
- PrP-specific PCR products were amplified (918 bp) from both P27 and P43 cells
  - Sequences matched GenBank #U29185
  - Insertion of 24 consecutive bases between bp 339 and 340 in both sequences
  - Insertion represents known octapeptide repeat
- **There was no evidence of infectious PrP^{SC}**

PrP Gene Product Protease Sensitivity

- Western blot assay employed that detects protease-resistant PrP gene product (Caughey, et.al, 1996, 1998)
- Assay performed on both P27 and P43 cells
  - Cells were cultured, PrP protein extracted, and subjected to Proteinase K digestion
  - Materials analyzed by Western blot using Mab 3F4 that recognizes both human and hamster PrP-res
  - Positive control = Hamster scrapies agent
  - Assay sensitivity $\leq$ 99% PrP sensitive to protease

PrP Gene Product Protease Sensitivity

Results:

Controls – All spiked samples and controls showed presence of protease-resistant PrP

P27 – No evidence of protease-resistant PrP

P43 – No evidence of protease-resistant PrP

Tumorigenicity Study

- Goal: Determine whether addition of ORF6 plasmid increases tumorigenic potential of 293-ORF6 cells over parental 293 cells
- Animals: Athymic Nude (nu/nu) mice, 20 mice per group
- Test Article: 293 parental and 293-ORF6 (P43) cells injected at 10^7, 10^5, and 10^3 cells/animal
- Positive control: HeLa at 10^7, 10^5 and 10^3 cells/animal
- Negative control: Syrian Hamster Embryo (SHE) at 10^7 cells/animal
- Study Duration: 140 days
- Endpoint: Histopathically-confirmed neoplasms

Tumorigenicity Study

Results:

HeLa Positive Control – Tumorigenic at 10^5 and 10^7 cells

SHE Negative Control – Not tumorigenic at 10^7 cells

293 Parent – Tumorigenic at 10^7 cells

293-ORF6 (P43) – Tumorigenic at 10^7 cells

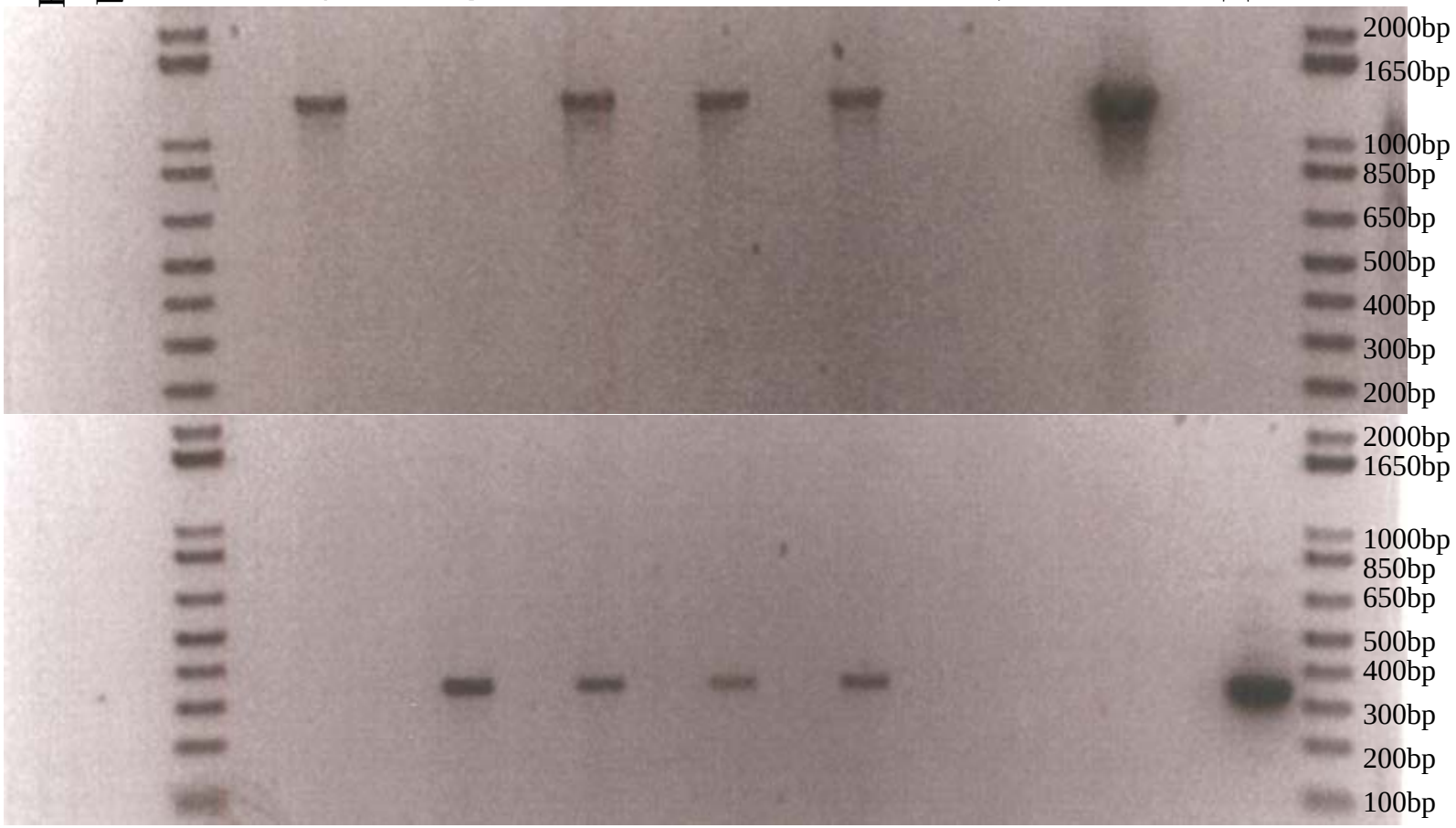
Conclusion: No difference observed

Oncogenicity Study

- Purpose: To further assure safety of vaccines planned with 293-ORF6 cells
- Design:
  - Test article: P43 cells
    - Cell lysate
    - Purified DNA
  - Species: Newborn rats, newborn hamsters
  - Administration: Subcutaneous, equivalent of 10^7 cells/animal
  - Controls:
    - Negative – MRC-5
    - Positive – None
- Study duration: 5 months
- Endpoint: Histopathically-confirmed neoplasms

293ORF6 Identity Assay Design

	E1	ORF6 EC
293 cells	+	-
293/ORF6 (P27)	+	+
293-ORF6 (P43)	+	+
A549 cells	-	-
J1-20 cells	-	+



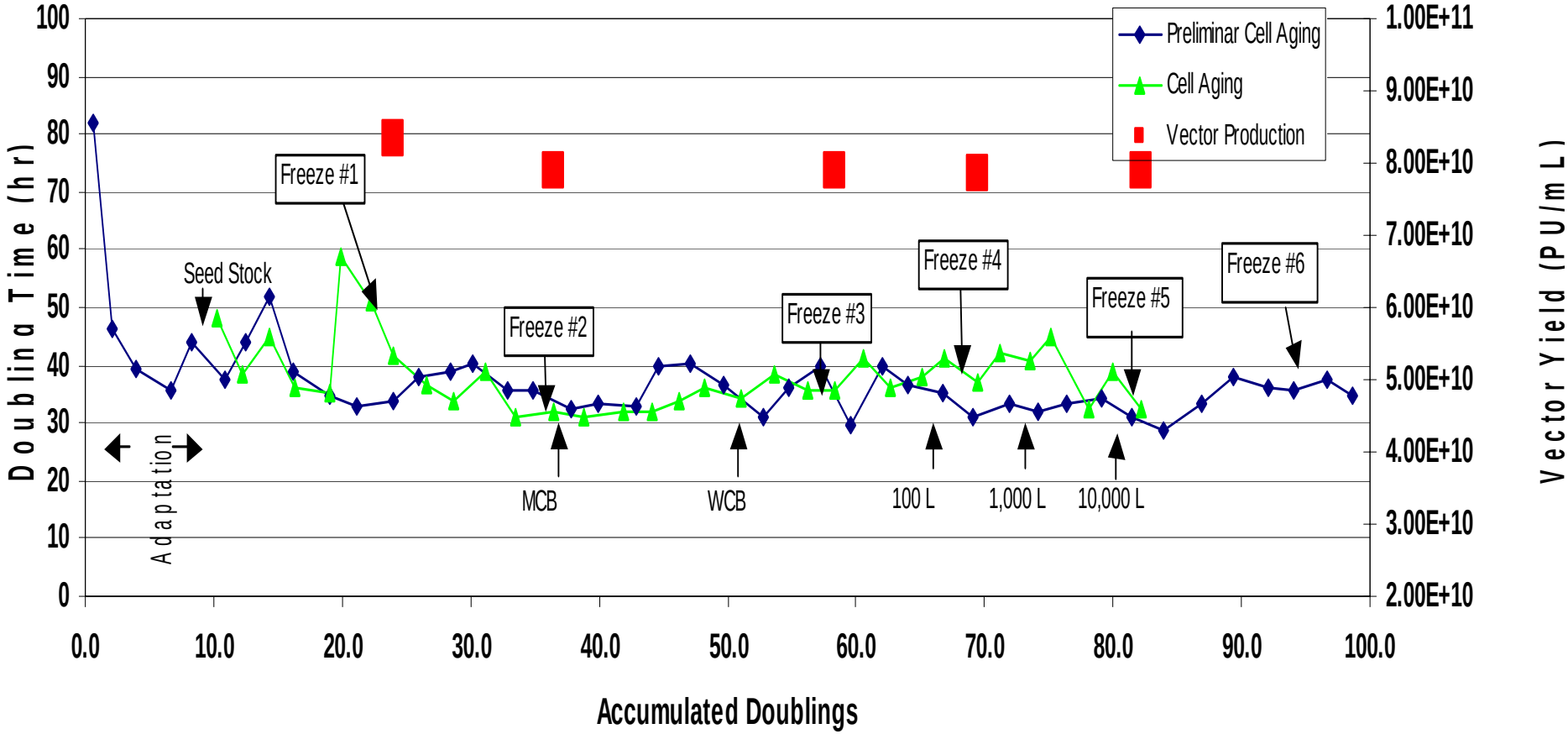
E1 PCR

1207bp

E4 PCR

307bp

Serum Free Suspension 293-ORF6 Cell Stability With Ad_{GV}EGR.TNF.11D



Summary & Conclusions

- Full characterization of GenVec's 293-ORF6 cell platform
  - Safety
  - Molecular characterization
  - Stability
- Submission of BMF to FDA
- Approval for use of 293-ORF6 cell line in the development of clinical vaccines

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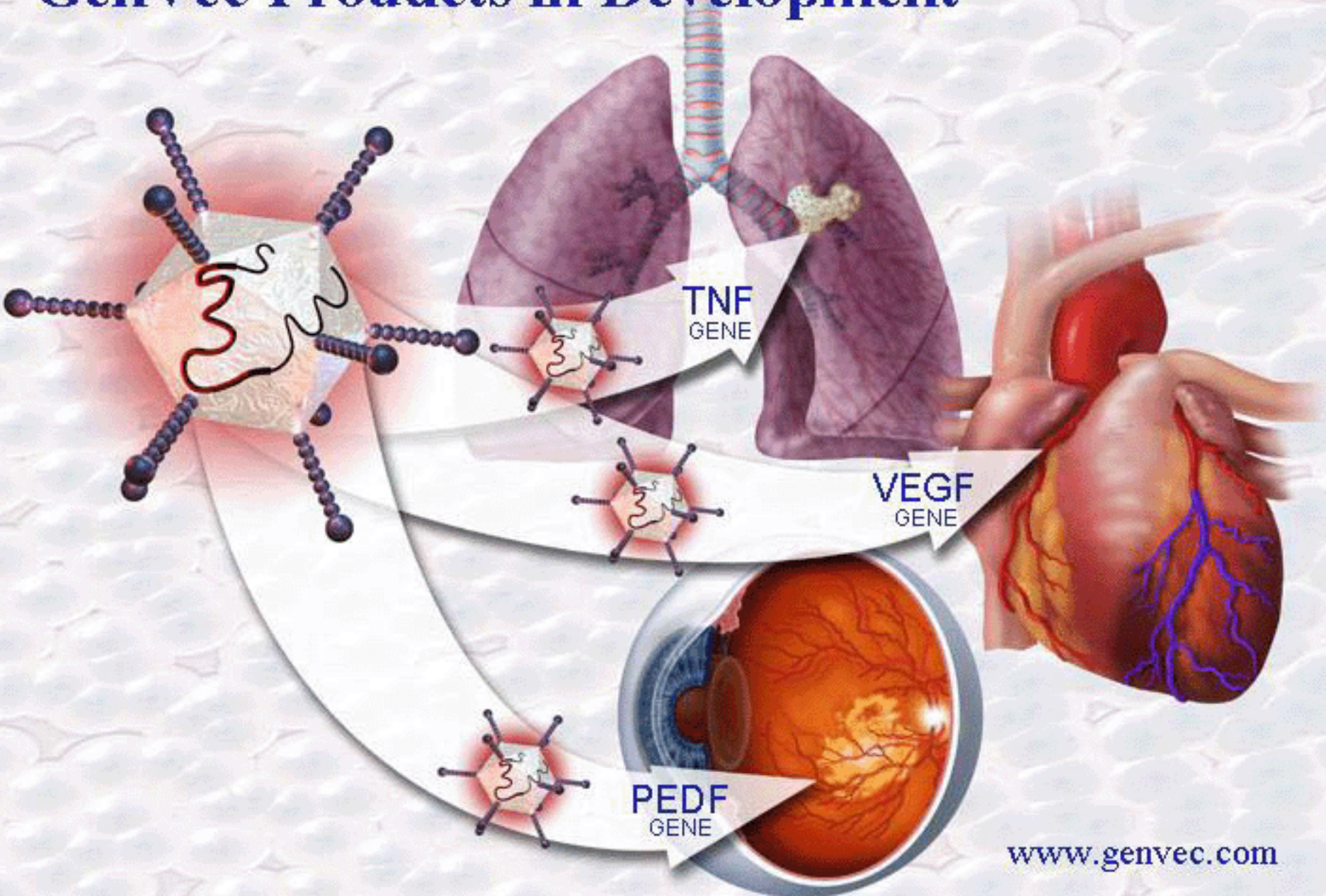
GenVec Products in Development



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